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Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Polyester synthase and a gene coding for the same

(57) The present invention relates to a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a sequence where in said amino acid sequence, one or more amino acids are deleted, replaced or added, said polypeptide having polyester synthase activity; a polyester synthase gene comprising DNA coding for said polypeptide; a recombinant vector comprising the gene; and a transformant transformed with the recombinant vector.

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Description

Field of the Invention

[0001] The present invention relates to polyester synthase, a gene coding for the enzyme, a recombinant vector containing the gene, a transformant transformed with the vector, and a process for producing polyester synthase by use of the transformant.

Background of the Invention

[0002] Polyesters (e.g. poly-3-hydroxyalkanoic acid) biosynthesized by microorganisms are biodegradable plastics with thermoplasticity ranging widely from rigid matter to viscoelastic rubber.

[0003] Poly-3-hydroxybutanoic acid (P(3HB)) is a typical polyester consisting of C4 monomer units, but it is a rigid and brittle polymeric material, so its application is limited. Accordingly, various polyesters such as P(3HB-co-3HV) having (P(3HB)) copolymerized with a C5 monomer unit (3HV) by adding propionic acid etc. to the medium have been prepared and examined to alter the physical properties of the polyester. On the other hand, polyesters consisting of at least C6 monomer units are soft polymeric materials having plasticity.

[0004] Polyester-synthesizing microorganisms are roughly divided into 2 groups, that is, those synthesizing polyesters with C3-5 monomer units and those synthesizing polyesters with C6-14 monomer units. The former microorganisms possess a polyester synthase using C3-5 monomer units as the substrate, while the latter microorganisms possess a polyester synthase using C6-14 monomer units as the substrate. Therefore, polyesters with different properties are synthesized by the respective microorganisms.

[0005] However, the respective polyesters from such known microorganisms are different in substrate specificity, so with one kind of enzyme given, polyesters (copolymers) having various monomer unit compositions adapted to the object of use are difficult to synthesize.

Summary of the Invention

[0006] The object of the present invention is to provide a polyester synthase preferably having specificity for monomer units having a wide range of carbon atoms as the substrate, a gene coding for the enzyme, a recombinant vector containing the gene, a transformant transformed with the vector, and a process for producing the polyester synthase by use of the transformant.

[0007] As a result of their eager research, the present inventors succeeded in cloning a polyester synthase gene from a microorganism belonging to the genus Pseudomonas isolated from soil, to arrive at the completion of the present invention.

[0008] That is, the present invention relates to a polypeptide comprising the amino acid sequence of SEQ ID NO:1, analogs, variants or fragments thereof, for example where one or more amino acids are deleted, replaced or added, said polypeptide having polyester synthase activity.

[0009] Further, the present invention relates to a polyester synthase gene comprising DNA coding for said polypeptide or variants or fragments thereof. The DNA coding for the protein with polyester synthase activity includes e.g. that of SEQ ID NO:2.

[0010] Further, the present invention relates to a polyester synthase gene comprising the nucleotide sequence of SEQ ID NO:3.

[0011] Further, the present invention relates to a recombinant vector comprising the polyester synthase gene.

[0012] Further, the present invention relates to a transformant transformed with said recombinant vector.

[0013] Further, the present invention relates to a process for producing polyester synthase wherein said transformant is cultured in a medium and polyester synthase is recovered from the resulting culture.

Detailed Description of the Invention

[0014] Hereinafter, the present invention is described in detail.

(1) Cloning of the polyester synthase gene

[0015] The polyester synthase gene of the present invention may be isolated from a microorganism belonging to the genus Pseudomonas.

[0016] First, genomic DNA is isolated from a strain having the polyester synthase gene. Such a strain includes e.g. Pseudomonas sp. Any known methods can be used for preparation of genomic DNA. For example, Pseudomonas sp.

is cultured in a bouillon medium and then its genomic DNA is prepared by the hexadecyl trimethyl ammonium bromide method (Current Protocols in Molecular Biology, vol. 1, page 2.4.3., John Wiley & Sons Inc., 1994).

[0017] The DNA obtained in this manner is partially digested with a suitable restriction enzyme (e.g. Sau3AI, BamHI, BglII etc.). It is then ligated into a vector dephosphorylated by treatment with alkaline phosphatase after cleavage with a restriction enzyme (e.g. BamHI, BglII etc.) to prepare a library.

[0018] Phage or plasmid capable of autonomously replicating in host microorganisms is used as the vector. The phage vector includes e.g. EMBL3, M13, λgt11 etc., and the plasmid vector includes e.g. pBR322, pUC18, and pBluescript II (Stratagene). Vectors capable of autonomously replicating in 2 or more host cells such as E. coli and Bacillus brevis, as well as various shuttle vectors, can also be used. Such vectors are also cleaved with said restriction enzymes so that their fragment can be obtained.

[0019] Conventional DNA ligase is used to ligate the resulting DNA fragment into the vector fragment. The DNA fragment and the vector fragment are annealed and then ligated to produce a recombinant vector.

[0020] To introduce the recombinant vector into a host microorganism, any known methods can be used. For example, if the host microorganism is E. coli, the calcium chloride method (Lederberg, E.M. et al., J. Bacteriol. 119, 1072 (1974)) and the electroporation method (Current Protocols in Molecular Biology, vol. 1, page 1.8.4 (1994)) can be used. If phage DNA is used, the in vitro packaging method (Current Protocols in Molecular Biology, vol. 1, page 5.7.1 (1994)) etc. can be adopted. In the present invention, an in vitro packaging kit (Gigapack II, produced by Stratagene etc.) may be used.

[0021] To obtain a DNA fragment containing the polyester synthase gene derived from Pseudomonas sp., a probe is then prepared. The amino acid sequences of some polyester synthases have already been known (Peoples, O. P. and Sinskey, A. J., J. Biol. Chem., 264, 15293 (1989); Huisman, G. W. et al., J. Biol. Chem., 266, 2191 (1991); Pieper, U. et al., FEMS Microbiol. Lett., 96, 73 (1992); Timm, A. and Steinbuchel, A., Eur. J. Biochem., 209, 15 (1992), etc.). Well-conserved regions are selected from these amino acid sequences, and nucleotide sequences coding for them are estimated to design oligonucleotides. Examples of such oligonucleotides include, but are not limited to, the sequence 5'-CC(G/C)CAGATCAACAAGTT(C/T)TA(C/G)GAC-3' (SEQ ID NO:4) reported by Timm, A. and Steinbuchel, A., Eur. J. Biochem., 209, 15 (1992).

[0022] Then, this synthetic oligonucleotide is labeled with a suitable reagent and used for colony hybridization of the above genomic DNA library (Current Protocols in Molecular Biology, vol. 1, page 6.0.3 (1994)).

[0023] The E. coli is screened by colony hybridization, and a plasmid is recovered from it using the alkaline method (Current Protocols in Molecular Biology, vol. 1, page 1.6.1 (1994)), whereby a DNA fragment containing the polyester synthase gene is obtained. The nucleotide sequence of this DNA fragment can be determined in e.g. an automatic nucleotide sequence analyzer such as 373A DNA sequencer (Applied Biosystems) using a known method such as the Sanger method (Molecular Cloning, vol. 2, page 13.3 (1989)).

[0024] After the nucleotide sequence was determined by the means described above, the gene of the present invention can be obtained by chemical synthesis or the PCR technique using genomic DNA as a template, or by hybridization using a DNA fragment having said nucleotide sequence as a probe.

(2) Preparation of transformant

[0025] The transformant of the present invention is obtained by introducing the recombinant vector of the present invention into a host compatible with the expression vector used in constructing said recombinant vector.

[0026] The host is not particularly limited insofar as it can express the target gene. Examples are bacteria such as microorganisms belonging to the genus Alcaligenes, microorganisms belonging to the genus bacillus, bacteria such as E. coli, yeasts such as the genera Saccharomyces, Candida etc., and animal cells such as COS cells, CHO cells etc.

[0027] If microorganisms belonging to the genus Alcaligenes or bacteria such as E. coli are used as the host, the recombinant DNA of the present invention is preferably constituted such that it contains a promoter, the DNA of the present invention, and a transcription termination sequence so as to be capable of autonomous replication in the host. The expression vector includes pLA2917 (ATCC 37355) containing replication origin RK2 and pJRD215 (ATCC 37533) containing replication origin RSF1010, which are replicated and maintained in a broad range of hosts.

[0028] The promoter may be any one if it can be expressed in the host. Examples are promoters derived from E. coli, phage etc., such as trp promoter, lac promoter, PL promoter, PR promoter and T7 promoter. The method of introducing the recombinant DNA into bacteria includes e.g. a method using calcium ions (Current Protocols in Molecular Biology, vol. 1, page 1.8.1 (1994)) and the electroporation method (Current Protocols in Molecular Biology, vol. 1, page 1.8.4 (1994)).

[0029] If yeast is used as the host, expression vectors such as YEp13, YCp50 etc. are used. The promoter includes e.g. gal 1 promoter, gal 10 promoter etc. To method of introducing the recombinant DNA into yeast includes e.g. the electroporation method (Methods. Enzymol., 194, 182-187 (1990)), the spheroplast method (Proc. Natl. Acad. Sci. USA, 84, 1929-1933 (1978)), the lithium acetate method (J. Bacteriol., 153, 163-168 (1983)) etc.

[0030] If animal cells are used as the host, expression vectors such as pcDNAI, pcDNAI/Amp (produced by Invitrogene) etc. are used. The method of introducing the recombinant DNA into animal cells includes e.g. the electroporation method, potassium phosphate method etc.

(3) Production of polyester synthase

[0031] Production of the polyester synthase of the present invention is carried out by culturing the transformant of the present invention in a medium, forming and accumulating the polyester synthase of the present invention in the culture (the cultured microorganism or the culture supernatant) and recovering the polyester synthase from the culture.

[0032] A conventional method used for culturing the host is also used to culture the transformant of the present invention.

[0033] The medium for the transformant prepared from bacteria such as E. coli etc. as the host includes complete medium or synthetic medium, e.g. LB medium, M9 medium etc. The transformant is aerobically cultured at a temperature ranging from 25 to 37 °C for 12 to 48 hours so that the polyester synthase is accumulated in the microorganism and then recovered.

[0034] The carbon source is essential for the growth of the microorganism and includes e.g. carbohydrates such as glucose, fructose, sucrose, maltose etc.

[0035] The nitrogen source includes e.g. ammonia, ammonium salts such as ammonium chloride, ammonium sulfate, ammonium phosphate etc., peptone, meat extract, yeast extract, corn steep liquor etc. The inorganic matter includes e.g. monopotassium phosphate, dipotassium phosphate, magnesium phosphate, magnesium sulfate, sodium chloride etc.

[0036] Culture is carried out usually under aerobic conditions with shaking at 25 to 37 °C for more than 2 hours after expression is induced. During culture, antibiotics such as ampicillin, kanamycin, ampicillin, tetracycline etc. may be added to the culture.

[0037] To culture the microorganism transformed with the expression vector using an inducible promoter, its inducer can also be added to the medium. For example, isopropyl-β-D-thiogalactopyranoside (IPTG), indoleacrylic acid (IAA) etc. can be added to the medium.

[0038] To culture the transformant from animal cells as the host, use is made of a medium such as RPMI-1640 or DMEM which may be supplemented with fetal bovine serum. Culture is carried out usually in 5 % CO_2 at 30 to 37°C for 1 to 7 days. During culture, antibiotics such as kanamycin, penicillin etc. may be added to the medium.

[0039] Purification of the polyester synthase can be performed by recovering the resulting culture by centrifugation (after disruption in the case of cells) and subjecting it to affinity chromatography, cation or anion exchange chromatography or gel filtration or to a suitable combination thereof.

[0040] Whether the resulting purified substance is the desired enzyme is confirmed by conventional methods such as SDS polyacrylamide gel electrophoresis, Western blotting etc.

Examples

[0041] Hereinafter, the present invention is described in more detail with reference to the Examples which however are not intended to limit the scope of the present invention.

(1) Cloning of the polyester synthase gene from Pseudomonas sp.

[0042] First, a genomic DNA library of Pseudomonas sp. was prepared.

[0043] Pseudomonas sp. JCM 10015 was cultured overnight in 100 ml bouillon medium (1 % meat extract, 1 % peptone, 0.5 % sodium chloride, pH 7.2) at 30 °C and then genomic DNA was obtained from the microorganism using the hexadecyl trimethyl ammonium bromide method (Current Protocols in Molecular Biology, vol. 1, page 2.4.3 (1994), John Wiley & Sons Inc.).

[0044] The resulting genomic DNA was partially digested with restriction enzyme Sau3AI. The vector plasmid used was cosmid vector pLA2917 (ATCC 37355). This plasmid was cleaved with restriction enzyme BglII and dephosphorylated (Molecular Cloning, vol. 1, page 5.7.2 (1989), Cold Spring Harbor Laboratory) and then ligated into the partially digested genomic DNA fragment by use of DNA ligase.

[0045] E. coli S17-1 was transformed with this ligated DNA fragment by the in vitro packaging method (Current Protocols in Molecular Biology, vol. 1, page 5.7.2 (1994)) whereby a genomic DNA library from Pseudomonas sp. was obtained.

[0046] To obtain a DNA fragment containing the polyester synthase gene from Pseudomonas sp., a probe was then prepared. An oligonucleotide consisting of the sequence 5'- CC(G/C)CAGATCAACAAGTT(C/T)TA(C/G)GAC-3' (SEQ ID NO:4) reported by Timm, A. and Steinbuchel, A., Eur. J. Biochem., 209, 15 (1992) was synthesized. This oligonu-

cleotide was labeled with digoxigenin using a DIG DNA labeling kit (Boehringer Mannheim) and used as a probe.

[0047] Using the probe thus obtained, E. coli carrying a plasmid containing the polyester synthase gene was isolated by colony hybridization from the genomic DNA library from Pseudomonas sp.

[0048] When Alcaligenes eutrophus PHB-4 (DSM541) and Pseudomonas putida GPpl04 (both of which are strains deficient in an ability to produce polyester) were transformed by the conjugation transfer method with the plasmid containing the polyester synthase gene, both the strains had a reverse ability to produce polyester and showed complementarity.

[0049] By recovering the plasmid from the E. coli, a DNA fragment containing the polyester synthase gene was obtained.

[0050] The nucleotide sequence of a PstI-XbaI fragment from this fragment was determined by the Sanger method.

[0051] As a result, the nucleotide sequence of the 1.8 kbp fragment shown in SEQ ID NO:3 was determined.

[0052] By further examining homology to this nucleotide sequence, the polyester synthase gene containing the nucleotide sequence (1680 bp) of SEQ ID NO:2 could be identified in this 1.8 kbp nucleotide sequence. The amino acid sequence encoded by SEQ ID NO:2 is shown in SEQ ID NO:1.

[0053] It should be understood that insofar as a protein containing the amino acid sequence of SEQ ID NO:1 or a sequence where in said amino acid sequence, one or more amino acids are deleted, replaced or added has polyester synthase activity, the gene (SEQ ID NO:2 or 3) containing DNA coding for said protein falls under the scope of the polyester synthase gene of the present invention.

[0054] Mutations such as deletion, replacement, addition etc. can be induced in the amino acid sequence or nucleotide sequence by the known site-direct mutagenesis method (e.g. Transfomer™ Site-Directed Mutagenesis Kit available from Toyobo).

(2) Preparation of E. coli transformant.

[0055] The 1.8 kb PstI-XbaI fragment containing the polyester synthase gene was ligated into the XbaI, PstI site of plasmid vector pBluescript II KS+. The resulting recombinant vector was transformed by the calcium chloride method into Escherichia coli DH5α. The resulting transformant was designated Escherichia coli PX18. By extracting the plasmid from this transformant, the 1.8 kb PstI-XbaI fragment containing the polyester synthase gene can be easily obtained. Escherichia coli PX18 has been deposited as FERM BP-6297 with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken 305-8566, Japan).

[0056] According to the present invention, there are provided a gene coding for polyester synthase, a recombinant vector containing the gene, and a transformant transformed with the vector. The gene of the present invention codes for a polyester synthase using monomers having a wide range of carbon atoms as the substrate, so it is useful in preparing copolymer polyesters having various physical properties.

[0057] The term "analog" as used herein, in relation to proteins or polypeptides of the present invention includes any peptidomimetic, that is, a chemical compound that possesses polyester synthase activity in a similar manner to the parent protein.

[0058] The term "variant" as used herein, in relation to proteins or polypeptides of the present invention includes any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid residues from or to the sequence providing that the resultant protein or polypeptide possesses polyester synthase activity.

[0059] The term "fragment" as used herein, in relation to proteins or polypeptides of the present invention includes any shorter forms of the polypeptides of the present invention that possesses polyester synthase activity in a similar manner to the parent protein.

[0060] The terms "variants" or "fragments" as used in relation to the DNA molecules of the present invention, have the meanings as given above (as appropriate to DNA) given that the DNA variant, fragment or derivative encodes a polypeptide that possesses polyester synthase activity in a similar manner to the parent protein.

Annex to the description

[0061]

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 559 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Ser Asn Lys Asn Ser Asp Asp Leu Asn Arg Gln Ala Ser Glu Asn
 1               5                  10                  15
Thr Leu Gly Leu Asn Pro Val Ile Gly Leu Arg Gly Lys Asp Leu Leu
            20                  25                  30
Thr Ser Ala Arg Met Val Leu Thr Gln Ala Ile Lys Gln Pro Ile His
        35                  40                  45
Ser Val Lys His Val Ala His Phe Gly Ile Glu Leu Lys Asn Val Met
    50                  55                  60
Phe Gly Lys Ser Lys Leu Gln Pro Glu Ser Asp Asp Arg Arg Phe Asn
65                  70                  75                  80
Asp Pro Ala Trp Ser Gln Asn Pro Leu Tyr Lys Arg Tyr Leu Gln Thr
                85                  90                  95
Tyr Leu Ala Trp Arg Lys Glu Leu His Asp Trp Ile Gly Asn Ser Lys
            100                 105                 110
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Leu Ser Glu Gln Asp Ile Asn Arg Ala His Phe Val Ile Thr Leu Met
115 120 125

Thr Glu Ala Met Ala Pro Thr Asn Ser Ala Ala Asn Pro Ala Ala Val
130 135 140

Lys Arg Phe Phe Glu Thr Gly Gly Lys Ser Leu Leu Asp Gly Leu Thr
145 150 155 160

His Leu Ala Lys Asp Leu Val Asn Asn Gly Gly Met Pro Ser Gln Val
165 170 175

Asp Met Gly Ala Phe Glu Val Gly Lys Ser Leu Gly Thr Thr Glu Gly
180 185 190

Ala Val Val Phe Arg Asn Asp Val Leu Glu Leu Ile Gln Tyr Arg Pro
195 200 205

Thr Thr Glu Gln Val His Glu Arg Pro Leu Leu Val Val Pro Pro Gln
210 215 220

Ile Asn Lys Phe Tyr Val Phe Asp Leu Ser Pro Asp Lys Ser Leu Ala
225 230 235 240

Arg Phe Cys Leu Ser Asn Asn Gln Gln Thr Phe Ile Val Ser Trp Arg
245 250 255

Asn Pro Thr Lys Ala Gln Arg Glu Trp Gly Leu Ser Thr Tyr Ile Asp
260 265 270

Ala Leu Lys Glu Ala Val Asp Val Val Ser Ala Ile Thr Gly Ser Lys
275 280 285

Asp Ile Asn Met Leu Gly Ala Cys Ser Gly Gly Ile Thr Cys Thr Ala
290 295 300

Leu Leu Gly His Tyr Ala Ala Leu Gly Glu Lys Lys Val Asn Ala Leu
305 310 315 320

Thr Leu Leu Val Ser Val Leu Asp Thr Thr Leu Asp Ser Gln Val Ala
325 330 335

Leu Phe Val Asp Glu Lys Thr Leu Glu Ala Ala Lys Arg His Ser Tyr

340 345 350

Gln Ala Gly Val Leu Glu Gly Arg Asp Met Ala Lys Val Phe Ala Trp

355 360 365

Met Arg Pro Asn Asp Leu Ile Trp Asn Tyr Trp Val Asn Asn Tyr Leu

370 375 380

Leu Gly Asn Glu Pro Pro Val Phe Asp Ile Leu Phe Trp Asn Asn Asp

385 390 395 400

Thr Thr Arg Leu Pro Ala Ala Phe His Gly Asp Leu Ile Glu Met Phe

405 410 415

Lys Asn Asn Pro Leu Val Arg Ala Asn Ala Leu Glu Val Ser Gly Thr

420 425 430

Pro Ile Asp Leu Lys Gln Val Thr Ala Asp Ile Tyr Ser Leu Ala Gly

435 440 445

Thr Asn Asp His Ile Thr Pro Trp Lys Ser Cys Tyr Lys Ser Ala Gln

450 455 460

Leu Phe Gly Gly Lys Val Glu Phe Val Leu Ser Ser Ser Gly His Ile

465 470 475 480

Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ser Arg Tyr Met Thr

485 490 495

Ser Thr Asp Met Pro Ala Thr Ala Asn Glu Trp Gln Glu Asn Ser Thr

500 505 510

Lys His Thr Asp Ser Trp Trp Leu His Trp Gln Ala Trp Gln Ala Glu

515 520 525

Arg Ser Gly Lys Leu Lys Lys Ser Pro Thr Ser Leu Gly Asn Lys Ala

530 535 540

Tyr Pro Ser Gly Glu Ala Ala Pro Gly Thr Tyr Val His Glu Arg

545 550 555

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1680 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGAGTAACA AGAATAGCGA TGACTTGAAT CGTCAAGCCT CGGAAAACAC CTTGGGGCTT 60

AACCCTGTCA TCGGCCTGCG TGGAAAAGAT CTGCTGACTT CTGCCCGAAT GGTTTTAACC 120

CAAGCCATCA AACAACCCAT TCACAGCGTC AAGCACGTCG CGCATTTTGG CATCGAGCTG 180

AAGAACGTGA TGTTTGGCAA ATCGAAGCTG CAACCGGAAA GCGATGACCG TCGTTTCAAC 240

GACCCCGCCT GGAGTCAGAA CCCACTCTAC AAACGTTATC TACAAACCTA CCTGGCGTGG 300

CGCAAGGAAC TCCACGACTG GATCGGCAAC AGCAAACTGT CCGAACAGGA CATCAATCGC 360

GCTCACTTCG TGATCACCCT GATGACCGAA GCCATGGCCC CGACCAACAG TGCGGCCAAT 420

CCGGCGGCGG TCAAACGCTT CTTCGAAACC GGCGGTAAAA GCCTGCTCGA CGGCCTCACA 480

CATCTGGCCA AGGACCTGGT AAACAACGGC GGCATGCCGA GCCAGGTGGA CATGGGCGCT 540

TTCGAAGTCG GCAAGAGTCT GGGGACGACT GAAGGTGCAG TGGTTTTCCG CAACGACGTC 600

CTCGAATTGA TCCAGTACCG GCCGACCACC GAACAGGTGC ATGAGCGACC GCTGCTGGTG 660

GTCCCACCGC AGATCAACAA GTTTTATGTG TTTGACCTGA GCCCGGATAA AAGCCTGGCG 720

CGCTTCTGCC TGAGCAACAA CCAGCAAACC TTTATCGTCA GCTGGCGCAA CCCGACCAAG 780

GCCCAGCGTG AGTGGGGTCT GTCGACTTAC ATCGATGCGC TCAAAGAAGC CGTCGACGTA 840

GTTTCCGCCA TCACCGGCAG CAAAGACATC AACATGCTCG GCGCCTGCTC CGGTGGCATT 900

ACCTGCACCG CGCTGCTGGG TCACTACGCC GCTCTCGGCG AGAAGAAGGT CAATGCCCTG 960

ACCCTTTTGG TCAGCGTGCT CGACACCACC CTCGACTCCC AGGTTGCACT GTTCGTCGAT 1020

GAGAAAACCC TGGAAGCTGC CAAGCGTCAC TCGTATCAGG CCGGCGTGCT GGAAGGCCGC 1080

GACATGGCCA AAGTCTTCGC CTGGATGCGC CCTAACGACC TGATCTGGAA CTACTGGGTC 1140

AACAACTACC TGCTGGGTAA CGAGCCACCG GTCTTCGACA TTCTTTTCTG GAACAACGAC 1200

ACCACCCGGT TGCCTGCTGC GTTCCACGGC GATCTGATCG AAATGTTCAA AAATAACCCA 1260

CTGGTGCGCG CCAATGCACT CGAAGTGAGC GGCACGCCGA TCGACCTCAA ACAGGTCACT 1320

GCCGACATCT ACTCCCTGGC CGGCACCAAC GATCACATCA CGCCCTGGAA GTCTTGCTAC 1380

AAGTCGGCGC AACTGTTCGG TGGCAAGGTC GAATTCGTGC TGTCCAGCAG TGGGCATATC 1440

CAGAGCATTC TGAACCCGCC GGGCAATCCG AAATCACGTT ACATGACCAG CACCGACATG 1500

CCAGCCACCG CCAACGAGTG GCAAGAAAAC TCAACCAAGC ACACCGACTC CTGGTGGCTG 1560

CACTGGCAGG CCTGGCAGGC CGAGCGCTCG GGCAAACTGA AAAAGTCCCC GACCAGCCTG 1620

GGCAACAAGG CCTATCCGTC AGGAGAAGCC GCGCCGGGCA CGTATGTGCA TGAACGTTAA 1680

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1826 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTGCAGTGCT CTCTGAACTA GAAAGCAACG TTGTGCAATT AACGGTCACC CGAGCAGTAG 60

TACCTGGCGG TTGCTGTGTG ACTACACAGC TGGTCCCGGT ACTCGTCTCA GGACAATGGA 120

GCGTCGTAGA TGAGTAACAA GAATAGCGAT GACTTGAATC GTCAAGCCTC GGAAAACACC 180

TTGGGGCTTA ACCCTGTCAT CGGCCTGCGT GGAAAAGATC TGCTGACTTC TGCCCGAATG 240

GTTTTAACCC AAGCCATCAA ACAACCCATT CACAGCGTCA AGCACGTCGC GCATTTTGGC 300

ATCGAGCTGA AGAACGTGAT GTTTGGCAAA TCGAAGCTGC AACCGGAAAG CGATGACCGT 360

CGTTTCAACG ACCCCGCCTG GAGTCAGAAC CCACTCTACA AACGTTATCT ACAAACCTAC 420

CTGGCGTGGC GCAAGGAACT CCACGACTGG ATCGGCAACA GCAAACTGTC CGAACAGGAC 480

ATCAATCGCG CTCACTTCGT GATCACCCTG ATGACCGAAG CCATGGCCCC GACCAACAGT 540

GCGGCCAATC CGGCGGCGGT CAAACGCTTC TTCGAAACCG GCGGTAAAAG CCTGCTCGAC 600

GGCCTCACAC ATCTGGCCAA GGACCTGGTA AACAACGGCG GCATGCCGAG CCAGGTGGAC 660

ATGGGCGCTT TCGAAGTCGG CAAGAGTCTG GGGACGACTG AAGGTGCAGT GGTTTTCCGC 720

AACGACGTCC TCGAATTGAT CCAGTACCGG CCGACCACCG AACAGGTGCA TGAGCGACCG 780

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CCGACCAAGG CCCAGCGTGA GTGGGGTCTG TCGACTTACA TCGATGCGCT CAAAGAAGCC 960

GTCGACGTAG TTTCCGCCAT CACCGGCAGC AAAGACATCA ACATGCTCGG CGCCTGCTCC 1020

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AACAACGACA CCACCCGGTT GCCTGCTGCG TTCCACGGCG ATCTGATCGA AATGTTCAAA 1380

AATAACCCAC TGGTGCGCGC CAATGCACTC GAAGTGAGCG GCACGCCGAT CGACCTCAAA 1440

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TCTTGCTACA AGTCGGCGCA ACTGTTCGGT GGCAAGGTCG AATTCGTGCT GTCCAGCAGT 1560

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TGGTGGCTGC ACTGGCAGGC CTGGCAGGCC GAGCGCTCGG GCAAACTGAA AAAGTCCCCG 1740

ACCAGCCTGG GCAACAAGGC CTATCCGTCA GGAGAAGCCG CGCCGGGCAC GTATGTGCAT 1800

GAACGTTAAG TTGTAGGCAG TCTAGA 1826

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCSCAGATCA ACAAGTTYTA SGAC 24

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Japan Science and Technology Corporation
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(ii) TITLE OF INVENTION: POLYESTER SYNTHASE AND A GENE CODING FOR THE SAME

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: JP 82965/1997
(B) FILING DATE: 01-APR-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 559 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Ser Asn Lys Asn Ser Asp Asp Leu Asn Arg Gln Ala Ser Glu Asn
 1               5                  10                  15
Thr Leu Gly Leu Asn Pro Val Ile Gly Leu Arg Gly Lys Asp Leu Leu
            20                  25                  30
Thr Ser Ala Arg Met Val Leu Thr Gln Ala Ile Lys Gln Pro Ile His
        35                  40                  45
Ser Val Lys His Val Ala His Phe Gly Ile Glu Leu Lys Asn Val Met
    50                  55                  60                  65
Phe Gly Lys Ser Lys Leu Gln Pro Glu Ser Asp Asp Arg Arg Phe Asn
                70                  75                  80
Asp Pro Ala Trp Ser Gln Asn Pro Leu Tyr Lys Arg Tyr Leu Gln Thr
            85                  90                  95
Tyr Leu Ala Trp Arg Lys Glu Leu His Asp Trp Ile Gly Asn Ser Lys
        100                 105                 110
Leu Ser Glu Gln Asp Ile Asn Arg Ala His Phe Val Ile Thr Leu Met
    115                 120                 125
Thr Glu Ala Met Ala Pro Thr Asn Ser Ala Ala Asn Pro Ala Ala Val
130                 135                 140                 145
Lys Arg Phe Phe Glu Thr Gly Gly Lys Ser Leu Leu Asp Gly Leu Thr
                150                 155                 160
His Leu Ala Lys Asp Leu Val Asn Asn Gly Gly Met Pro Ser Gln Val
            165                 170                 175
Asp Met Gly Ala Phe Glu Val Gly Lys Ser Leu Gly Thr Thr Glu Gly
        180                 185                 190
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Ala Val Val Phe Arg Asn Asp Val Leu Glu Leu Ile Gln Tyr Arg Pro
    195                 200                 205
Thr Thr Glu Gln Val His Glu Arg Pro Leu Leu Val Val Pro Pro Gln
210                 215                 220                 225
Ile Asn Lys Phe Tyr Val Phe Asp Leu Ser Pro Asp Lys Ser Leu Ala
                230                 235                 240
Arg Phe Cys Leu Ser Asn Asn Gln Gln Thr Phe Ile Val Ser Trp Arg
            245                 250                 255
Asn Pro Thr Lys Ala Gln Arg Glu Trp Gly Leu Ser Thr Tyr Ile Asp
        260                 265                 270
Ala Leu Lys Glu Ala Val Asp Val Val Ser Ala Ile Thr Gly Ser Lys
    275                 280                 285
Asp Ile Asn Met Leu Gly Ala Cys Ser Gly Gly Ile Thr Cys Thr Ala
290                 295                 300                 305
Leu Leu Gly His Tyr Ala Ala Leu Gly Glu Lys Lys Val Asn Ala Leu
                310                 315                 320
Thr Leu Leu Val Ser Val Leu Asp Thr Thr Leu Asp Ser Gln Val Ala
            325                 330                 335
Leu Phe Val Asp Glu Lys Thr Leu Glu Ala Ala Lys Arg His Ser Tyr
        340                 345                 350
Gln Ala Gly Val Leu Glu Gly Arg Asp Met Ala Lys Val Phe Ala Trp
    355                 360                 365
Met Arg Pro Asn Asp Leu Ile Trp Asn Tyr Trp Val Asn Asn Tyr Leu
370                 375                 380                 385
Leu Gly Asn Glu Pro Pro Val Phe Asp Ile Leu Phe Trp Asn Asn Asp
                390                 395                 400
Thr Thr Arg Leu Pro Ala Ala Phe His Gly Asp Leu Ile Glu Met Phe
            405                 410                 415
Lys Asn Asn Pro Leu Val Arg Ala Asn Ala Leu Glu Val Ser Gly Thr
        420                 425                 430
Pro Ile Asp Leu Lys Gln Val Thr Ala Asp Ile Tyr Ser Leu Ala Gly
    435                 440                 445
Thr Asn Asp His Ile Thr Pro Trp Lys Ser Cys Tyr Lys Ser Ala Gln
450                 455                 460                 465
Leu Phe Gly Gly Lys Val Glu Phe Val Leu Ser Ser Ser Gly His Ile
                470                 475                 480
Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ser Arg Tyr Met Thr
            485                 490                 495
Ser Thr Asp Met Pro Ala Thr Ala Asn Glu Trp Gln Glu Asn Ser Thr
        500                 505                 510
Lys His Thr Asp Ser Trp Trp Leu His Trp Gln Ala Trp Gln Ala Glu
    515                 520                 525
Arg Ser Gly Lys Leu Lys Lys Ser Pro Thr Ser Leu Gly Asn Lys Ala
530                 535                 540                 545
Tyr Pro Ser Gly Glu Ala Ala Pro Gly Thr Tyr Val His Glu Arg
                550                 555                 560
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1680 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
ATGAGTAACA AGAATAGCGA TGACTTGAAT CGTCAAGCCT CGGAAAACAC CTTGGGGCTT   60
AACCCTGTCA TCGGCCTGCG TGGAAAAGAT CTGCTGACTT CTGCCCGAAT GGTTTTAACC  120
CAAGCCATCA AACAACCCAT TCACAGCGTC AAGCACGTCG CGCATTTTGG CATCGAGCTG  180
AAGAACGTGA TGTTTGGCAA ATCGAAGCTG CAACCGGAAA GCGATGACCG TCGTTTCAAC  240
GACCCCGCCT GGAGTCAGAA CCCACTCTAC AAACGTTATC TACAAACCTA CCTGGCGTGG  300
CGCAAGGAAC TCCACGACTG GATCGGCAAC AGCAAACTGT CCGAACAGGA CATCAATCGC  360
GCTCACTTCG TGATCACCCT GATGACCGAA GCCATGGCCC CGACCAACAG TGCGGCCAAT  420
CCGGCGGCGG TCAAACGCTT CTTCGAAACC GGCGGTAAAA GCCTGCTCGA CGGCCTCACA  480
CATCTGGCCA AGGACCTGGT AAACAACGGC GGCATGCCGA GCCAGGTGGA CATGGGCGCT  540
TTCGAAGTCG GCAAGAGTCT GGGGACGACT GAAGGTGCAG TGGTTTTCCG CAACGACGTC  600
CTCGAATTGA TCCAGTACCG GCCGACCACC GAACAGGTGC ATGAGCGACC GCTGCTGGTG  660
GTCCCACCGC AGATCAACAA GTTTTATGTG TTTGACCTGA GCCCGGATAA AAGCCTGGCG  720
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CGCTTCTGCC TGAGCAACAA CCAGCAAACC TTTATCGTCA GCTGGCGCAA CCCGACCAAG 780
GCCCAGCGTG AGTGGGGTCT GTCGACTTAC ATCGATGCGC TCAAAGAAGC CGTCGACGTA 840
GTTTCCGCCA TCACCGGCAG CAAAGACATC AACATGCTCG GCGCCTGCTC CGGTGGCATT 900
ACCTGCACCG CGCTGCTGGG TCACTACGCC GCTCTCGGCG AGAAGAAGGT CAATGCCCTG 960
ACCCTTTTGG TCAGCGTGCT CGACACCACC CTCGACTCCC AGGTTGCACT GTTCGTCGAT 1020
GAGAAAACCC TGGAAGCTGC CAAGCGTCAC TCGTATCAGG CCGGCGTGCT GGAAGGCCGC 1080
GACATGGCCA AAGTCTTCGC CTGGATGCGC CCTAACGACC TGATCTGGAA CTACTGGGTC 1140
AACAACTACC TGCTGGGTAA CGAGCCACCG GTCTTCGACA TTCTTTTCTG GAACAACGAC 1200
ACCACCCGGT TGCCTGCTGC GTTCCACGGC GATCTGATCG AAATGTTCAA AAATAACCCA 1260
CTGGTGCGCG CCAATGCACT CGAAGTGAGC GGCACGCCGA TCGACCTCAA ACAGGTCACT 1320
GCCGACATCT ACTCCCTGGC CGGCACCAAC GATCACATCA CGCCCTGGAA GTCTTGCTAC 1380
AAGTCGGCGC AACTGTTCGG TGGCAAGGTC GAATTCGTGC TGTCCAGCAG TGGGCATATC 1440
CAGAGCATTC TGAACCCGCC GGGCAATCCG AAATCACGTT ACATGACCAG CACCGACATG 1500
CCAGCCACCG CCAACGAGTG GCAAGAAAAC TCAACCAAGC ACACCGACTC CTGGTGGCTG 1560
CACTGGCAGG CCTGGCAGGC CGAGCGCTCG GGCAAACTGA AAAAGTCCCC GACCAGCCTG 1620
GGCAACAAGG CCTATCCGTC AGGAGAAGCC GCGCCGGGCA CGTATGTGCA TGAACGTTAA 1680

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1826 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTGCAGTGCT CTCTGAACTA GAAAGCAACG TTGTGCAATT AACGGTCACC CGAGCAGTAG 60
TACCTGGCGG TTGCTGTGTG ACTACACAGC TGGTCCCGGT ACTCGTCTCA GGACAATGGA 120
GCGTCGTAGA TGAGTAACAA GAATAGCGAT GACTTGAATC GTCAAGCCTC GGAAAACACC 180
TTGGGGCTTA ACCCTGTCAT CGGCCTGCGT GGAAAAGATC TGCTGACTTC TGCCCGAATG 240
GTTTTAACCC AAGCCATCAA ACAACCCATT CACAGCGTCA AGCACGTCGC GCATTTTGGC 300
ATCGAGCTGA AGAACGTGAT GTTTGGCAAA TCGAAGCTGC AACCGGAAAG CGATGACCGT 360
CGTTTCAACG ACCCCGCCTG GAGTCAGAAC CCACTCTACA AACGTTATCT ACAAACCTAC 420
CTGGCGTGGC GCAAGGAACT CCACGACTGG ATCGGCAACA GCAAACTGTC CGAACAGGAC 480
ATCAATCGCG CTCACTTCGT GATCACCCTG ATGACCGAAG CCATGGCCCC GACCAACAGT 540
GCGGCCAATC CGGCGGCGGT CAAACGCTTC TTCGAAACCG GCGGTAAAAG CCTGCTCGAC 600
GGCCTCACAC ATCTGGCCAA GGACCTGGTA AACAACGGCG GCATGCCGAG CCAGGTGGAC 660
ATGGGCGCTT TCGAAGTCGG CAAGAGTCTG GGGACGACTG AAGGTGCAGT GGTTTTCCGC 720
AACGACGTCC TCGAATTGAT CCAGTACCGG CCGACCACCG AACAGGTGCA TGAGCGACCG 780
CTGCTGGTGG TCCCACCGCA GATCAACAAG TTTTATGTGT TTGACCTGAG CCCGGATAAA 840
AGCCTGGCGC GCTTCTGCCT GAGCAACAAC CAGCAAACCT TTATCGTCAG CTGGCGCAAC 900
CCGACCAAGG CCCAGCGTGA GTGGGGTCTG TCGACTTACA TCGATGCGCT CAAAGAAGCC 960
GTCGACGTAG TTTCCGCCAT CACCGGCAGC AAAGACATCA ACATGCTCGG CGCCTGCTCC 1020
GGTGGCATTA CCTGCACCGC GCTGCTGGGT CACTACGCCG CTCTCGGCGA GAAGAAGGTC 1080
AATGCCCTGA CCCTTTTGGT CAGCGTGCTC GACACCACCC TCGACTCCCA GGTTGCACTG 1140
TTCGTCGATG AGAAAACCCT GGAAGCTGCC AAGCGTCACT CGTATCAGGC CGGCGTGCTG 1200
GAAGGCCGCG ACATGGCCAA AGTCTTCGCC TGGATGCGCC CTAACGACCT GATCTGGAAC 1260
TACTGGGTCA ACAACTACCT GCTGGGTAAC GAGCCACCGG TCTTCGACAT TCTTTTCTGG 1320
AACAACGACA CCACCCGGTT GCCTGCTGCG TTCCACGGCG ATCTGATCGA AATGTTCAAA 1380
AATAACCCAC TGGTGCGCGC CAATGCACTC GAAGTGAGCG GCACGCCGAT CGACCTCAAA 1440
CAGGTCACTG CCGACATCTA CTCCCTGGCC GGCACCAACG ATCACATCAC GCCCTGGAAG 1500
TCTTGCTACA AGTCGGCGCA ACTGTTCGGT GGCAAGGTCG AATTCGTGCT GTCCAGCAGT 1560
GGGCATATCC AGAGCATTCT GAACCCGCCG GGCAATCCGA AATCACGTTA CATGACCAGC 1620
ACCGACATGC CAGCCACCGC CAACGAGTGG CAAGAAAACT CAACCAAGCA CACCGACTCC 1680
TGGTGGCTGC ACTGGCAGGC CTGGCAGGCC GAGCGCTCGG GCAAACTGAA AAAGTCCCCG 1740
ACCAGCCTGG GCAACAAGGC CTATCCGTCA GGAGAAGCCG CGCCGGGCAC GTATGTGCAT 1800
GAACGTTAAG TTGTAGGCAG TCTAGA @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ 1826

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCSCAGATCA ACAAGTTYTA SGAC 24

Claims

1. A polypeptide comprising the amino acid sequence of SEQ ID NO:1 or analogs, variants or fragments thereof.
2. A polyester synthase gene comprising DNA coding for a protein containing the amino acid sequence of SEQ ID NO:1 or an analog, variant or fragment thereof.
3. A polyester synthase gene according to claim 2, wherein the DNA coding for the protein with polyester synthase activity is that of SEQ ID NO:2.
4. A polyester synthase gene comprising the nucleotide sequence of SEQ ID NO:3.
5. A recombinant vector comprising the polyester synthase gene of any one of claims 2 to 4.
6. A transformant transformed with the recombinant vector of claim 4.
7. A process for producing polyester synthase, wherein the transformant of claim 6 is cultured in a medium and polyester synthase is recovered from the resulting culture.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 30 2554

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	WO 97 22711 A (REGENTS OF THE UNIVERSITY OF MINNESOTA) 26 June 1997 * page 3, line 10 - page 7, line 31; figures 23J,K; example 3 *	1,2,5-7	C12N15/52 C12N15/60 C12N9/88 C12N15/74 C12N1/21
X	WO 91 00917 A (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 24 January 1991	1,2,5-7	
Y	* page 5, line 9 - line 30 * * page 7, line 1 - line 5 * * page 36, line 15 - page 40, line 5; figures 5,6 *	3,4	
D,X	TIMM, A. AND STEINBÜCHEL, A.: "Cloning and molecular analysis of the poly(3-hydroxyalkanoic acid) gene locus of Pseudomonas aeruginosa PAO1" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 209, no. 1, October 1992, pages 15-30, XP002087430	1,2,5-7	
Y	* page 16, left-hand column, line 12 - line 28 * * page 18, left-hand column, line 24 - page 19, right-hand column, line 17 * * page 25 - page 28 * 'Discussion' * figures 1,2 *	3,4	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N
D,X	HUISMAN, G.W. ET AL.: "Metabolism of poly(3-hydroxyalkanoates) (PHAs) by Pseudomonas oleovorans" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 4, 5 February 1991, pages 2191-2198, XP002087431	1,2,5-7	
Y	* page 2192 - page 2197 * 'Results' and 'Discussion' * figure 2 *	3,4	

-/--

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	10 December 1998	Donath, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 30 2554

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	TIMM, A. ET AL.: "A general method for identification of polyhydroxyalkanoic acid synthase genes from pseudomonads belonging to the rRNA homology group I" APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 40, no. 5, January 1994, pages 669-675, XP002087432 * page 670 - page 671 * 'Identification of PHA synthase genes' and 'Cloning of the PHA synthase genes' * page 673 - page 674 * 'Discussion' ---	1-7	
Y	STEINBÜCHEL, A. ET AL.: "Molecular basis for biosynthesis and accumulation of polyhydroxyalkanoic acids in bacteria" FEMS MICROBIOLOGY REVIEWS, vol. 103, no. 2-4, December 1992, pages 217-230, XP002087433 * page 219 - page 224 * '4.Cloning of PHA-biosynthetic genes' * page 228 - page 229 * 'Conclusions' -----	1-7	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	10 December 1998	Donath, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 98 30 2554

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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10-12-1998

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9722711 A	26-06-1997	EP 0870053 A	14-10-1998
WO 9100917 A	24-01-1991	AT 172497 T	15-11-1998
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		US 5250430 A	05-10-1993

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82